Example 3—TDO2 Enzyme Inhibition Assay

[0940] The effect of compounds on TDO2 enzymatic activity was assess by use of an hTDO2 assay kit (Netherlands Translational Research Center B.V., Cat #NTRC-hTDO-1K). TDO2 enzyme and tryptophan substrate are diluted in assay buffer. Compounds are tested at 8 consecutive 3-fold dilutions starting from 30 μM. 267 nL of compound is added per well of a 384-well plate followed by addition of 10 μL of assay buffer. TDO2 enzyme is added at a final concentration of 50 nM and pre-incubated for 60 min at room temperature in the dark. Tryptophan substrate solution is then added at final concentration of 200 μM to all wells and the plate incubated for 15 min at RT in the dark. Finally NFK-green reagent is added at ⅓ of total reaction volume and incubated for 4 h at RT in the dark. After incubation fluorescence is measured at Ex405 nm-Em535 nm using EnVision plate reader.

[0941] IC50 values are determined using GraphPad Prism software 5.04 for Windows by plotting percent of inhibition against log 10 concentrations of compounds using a four-parametric sigmoidal curve with a variable slope.

TABLE 4

Inhibition of TDO2 activity by a selection of compounds of invention 1

Compound	TDO2 Biochemical assay IC50	Compound	TDO2 Biochemical assay IC50
3	>30	130	>30
21	>30	154	>30
137	>30	185	>30
144	>30	74	>30
80	>30	104	>30
83	>30	123	>30
147	>30	105	>30
90	>30	109	6.0
92	>30	110	>30
102	>30	158	6.4
111	4.8/2.2	162	>30

Example 4—Effect of Compounds on IDO1 Expression in SK-OV-3 Cell Line

[0942] SK-OV-3 cells are seeded in 24-well plates in McCoy's 5A medium supplemented with 10% FBS at density of 250000 cells per well and incubated overnight at 37° C., 5% CO2, 95% humidity. The following day growth medium is replaced with DMEM F-12 medium containing 6 mg/mL L-tryptophan. Compounds are added to the wells and the plate is incubated for 24 h at 37° C., 5% CO2, 95% humidity. At the end of incubation medium is removed, cells are washed and then lysed in RIPA buffer. Protein concentrations in the samples are determined using BCA method (Thermo Scientific) and adjusted to 0.5 mg/mL. Western analysis is performed on the Wes system (Protein Simple) using IDO1 (Cell Signalling; Cat #12006S) and GAPDH (Abcam, Cat #ab9485) antibodies. Expression of IDO1 is normalised to GAPDH.

Example 5—Effect of Compounds on Tryptophan Catabolism in Monocyte-Derived Dendritic Cells

[0943] Peripheral blood mononuclear cells are isolated from buffy coats from healthy volunteers by Lymphoprep density gradient centrifugation, followed by lysis of residual erythrocytes with isotonic buffer solution of ammonium chloride. CD14+ cells are isolated by positive selection using MACS® technology and CD14 MicroBeads (Miltenyi Biotec) according to manufacturer's instructions. The isolated monocytes are differentiated into dendritic cells by incubation for 5 days in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 50 ng/mL GM-CSF and 35 ng/mL IL-4. After 5 days the DCs are seeded in 96-well plates in DMEM supplemented with 10% charcoal stripped FBS, GM-CSF and IL-4. The following day cells are triggered with LPS and IFNγ. After 24 h of incubation medium is replaced with DMEM F-12 medium supplemented with 6 mg/mL L-tryptophan and 10% charcoal stripped FBS. Compounds are added to the cells and incubated for 24 h at 37° C., 5% CO2, 95% humidity. Compounds are tested at 7 consecutive 4-fold dilutions starting from 30 μM in duplicate.

[0944] At the end of incubation concentration of kynurenine in cell supernatants is determined by LC-MS/MS. In parallel, cell viability is assessed by measurement of ATP by use of CellTiter Glo assay (Promega) according to manufacturer's protocol.

[0945] Inhibition of Kyn production is calculated using the following formula:

(1−(Kyn conc. cmpd.−Kyn conc. medium)/(Kyn conc. medium with cells−Kyn conc. medium)) ·100

[0946] IC50 values are determined using GraphPad Prism software 5.04 for Windows, by plotting percent of inhibition against log 10 concentrations of compounds using a four-parametric sigmoidal curve with a variable slope.

[0947] Cell viability results are analysed in Microsoft Excel and expressed as fold change over untreated control.

TABLE 5

inhibition of kynurenine production and cytotoxicity of compounds of the invention in dendritic cells

Compound	Kynurenine IC50	Cytotoxicity at 30 uM
26	0.10	19
56	0.25	94
137	0.040	88
139	0.27	77
140	0.32	92
141	0.47	90
144	0.38	109
146	0.12	103
80	0.026	112
82	0.30	85
83	0.13	89
84	0.18	108
147	0.070	122
148	0.15	103
89	0.065	81
90	0.066	67
91	0.036	71

Example 6—Tumour Cell Killing Assay

[0948] Human PBMCs from healthy donors are prepared from buffy coat and cryopreserved. For the killing assay, on day −1, NucLight™ Red transfected SK-OV-3 ovarian cancer cells are seeded in to a Flat-bottomed 96-well plate at 2×103 cells/well (100 μL per well) and incubated in the Incucyte Zoom® overnight. The following day (day 0) media is aspirated from wells containing SK-OV-3 cells.